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EFFECT OF DIFFERENT SOURCES OF DIETARY FIBER ON GROWTH, APPARENT DIGESTIBILITY OF PROTEIN, FAT AND ENERGY AND PROTEIN UTILIZATION IN RATS

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ABSTRACT

The study of the effects of dietary fibers on nutrients metabolism is complicated by the fact that the dietary fiber is not a homogeneous compound but consists of a variety of substances, including cellulose, hemicellulose, pectin, mucilage, gums, algal polysaccharides and lignin. Therefore, in the present study we would like to determine the effect of gum Arabic (GA), carrgeenan (CR) compared to cellulose (CE) as a control on protein indices, fat and energy metabolism in rats. Wistar strain male rats (36) weighing 45-50gm were randomly assigned to six experimental groups, six animals to each group and housed individually in stainless steel metabolic cages. Six diets, three of them contain GA, CR or CE (5.2%) and casein to give 10% protein. Food intake, weight gain (WG), apparent digestibility of protein (APD), fat (AFD) and energy (AED), protein biological value (PBV), energy and amino acids apparent digestibility were determined. There was no significant ($P \leq 0.05$) difference in food intake between the groups. There was a significant ($P \leq 0.05$) difference in WG between gum arabic group and other two groups. Addition of cellulose gave higher APD and that of gum arabic gave higher AFD and AED. There was no significant ($P \leq 0.05$) difference in true protein digestibility between the groups but diets with CE or CR slightly gave high values. The biological value was higher for GA diet and no significant ($P \leq 0.05$) difference between the groups in net protein utilization. No significant ($P \leq 0.05$) difference in urine, fecal, intake and metabolic energy among the groups. The apparent amino acids digestibility varied significantly ($P \leq 0.05$) within and among the groups. The results indicate that gum Arabic as a source of dietary fiber compared to carrgeenan and cellulose did not affect food intake and in the meantime improved nutrients metabolism in rats.

Key words: gum arabic, carageenan, cellulose, apparent digestibility, energy, rats.

INTRODUCTION

Polysaccharides used in manufactured products for improving structure and stability were found to reduce both *in vivo* and *in vitro* protein digestibility and nutrient absorption in most cases (El Kossori *et al*. 2000; Mouecoucou *et al*. 2004). According to El Kossori *et al*. (2000), this is due to a reduction in enzymatic activity and an increase in viscosity or to interactions that could occur between proteins and polysaccharides (El Kossori *et al*. 2000; Mouecoucou *et al*. 2004). In solution, these interactions depend on pH and ionic strength, environment, structure and concentration of different biopolymers (Pego *et al*. 2003).

The effect of polysaccharides on protein digestibility was often assessed *in vitro* in closed systems or in dialysis bags with a MW cut-off (MWCO) of 1000 Da (El Kossori *et al.* 2000; Mouecoucou *et al.* 2004), but never increased intestinal permeability, where high molecular weight peptides cross the intestinal barrier. The decrease of protein digestibility by polysaccharides is often explained by the interactions between these two macromolecules that prevent the protein hydrolysis. Dietary fibres include not only material such as wheat bran which is removed during the refinement of wheat, but also viscous soluble polysaccharides such as pectin, locust bean gum, carrageenan, alginic acid and gum arabic.

Gum Arabic is primarily indigestible to both humans and animals. It is not degraded in the small intestine, but fermented in the large intestine by microorganisms to short-chain fatty acids, particularly propionic acid (Ali and Blunden 2009). Such degradation products are absorbed in the human colon and subsequently utilized energetically in metabolism. The study of the effects of dietary fibres on digestion is complicated by the fact that the dietary fibre is not a homogeneous compound but consists of a variety of substances, including cellulose, hemicellulose, pectin, mucilage, gums, algal polysaccharides and lignin. Results found by Aslaksen *et al.* (2007), however, indicate a negative effect of dietary cellulose levels on lipid digestibility in Atlantic salmon (*Salmo salar*).

The study of the effects of dietary fibers on nutrients metabolism is complicated by the fact that the dietary fiber is not a homogeneous compound but consists of a variety of substances, including cellulose, hemicellulose, pectin, mucilage, gums, algal polysaccharides and lignin. Moreover, most of the research investigated the effects of dietary fibers on nutrients metabolism is carried out on raw samples. Therefore, in the present study we would like to study the effect of pure samples of gum Arabic (GA), carrgeenan (CR) compared to cellulose (CE) as a control on protein indices, fat and energy metabolism in rats.

MATERIALS AND METHODS

Materials: Cellulose, gum Arabic and carageenan were obtained from local market in A Riyadh, Saudi Arabia. The gum Arabic was cleaned manually and ground in hammer mill to fine powder. The carageenan was purchased in powder form, and then stored refrigerated until use in a plastic container. All chemicals use in this study were of reagent grade.

Rats' diets: Wistar strain male rats (36) weighing 45-50gm were randomly assigned to six experimental groups, six animals to each group and housed individually in stainless steel metabolic cages. Six diets three of them containing cellulose, gum arabic or carrageenan (5.2%) and casein to give 10% protein. To determine metabolic endogenous nitrogen the remaining three diets contained the three fiber sources and egg protein (4%). The diet was prepared according to American Institute of Nutrition specification (Table 1). Briefly the diets comprised of 11.6% casein supplemented with 0.1% methionine or 4.92% egg protein, 9% sucrose5.2% corn oil, 5.2 fiber, 1% vitamin mix 3.5% mineral mix and corn starch. Each animal was provided with approximately 150 mg nitrogen (10 gm diet/day) and daily feed intake was recorded for each animal. The experiment lasted 9 days, with a preliminary feeding period of four days. Collection of feces and urine was carried out for a balance period of five days.

Protein and fat determination: The protein and fat were estimated according to the method described by the AOAC (1995).

Energy determination: The total and fecal energy were estimated using calorimetric pump (Ballistic Bomb Callenkamp Calorimeter, CBB. USA).

Nitrogen balance assay: The procedure described by Eggum (1973) was used to assess the effect of gum arabic and carrageenan (cellulose as a reference) on apparent protein digestibility (APD), apparent energy digestibility (AED) and apparent fat digestibility (AFD), metabolizable energy (ME), true protein digestibility (TPD), biological value (BV), net protein utilization (NPU).

The following equations were used to calculate the parameters of the diets:

$$APD\% = \frac{\text{N Intake} - \text{Fecal N}}{\text{N intake}} \text{X100}$$

$$AED\% = \frac{\text{Gross energy intake} - \text{Fecal gross energy}}{\text{Gross energy intake}} \text{X100}$$

$$AFD\% = \frac{\text{Fat intake} - \text{Fecal fat}}{\text{Fat intake}} \text{X100}$$

ME = Gross energy intake – (gross energy in urine + gross energy in feces)

$$TPD\% \ \frac{\text{N intake} - (\text{fecal N- metabolic fecal N})}{\text{N intake}} \text{X100}$$

$$BV \ \frac{\text{N intake} - (\text{fecal N- metabolic fecal N}) - (\text{urine N} - \text{metabolic urine N}}{\text{N intake} - (\text{fecal N - metabolic fecal N})}$$

NPU = BV X TPD

Amino acid analysis: The amino acids composition was determined according to the official methods using high-performance liquid chromatography (HPLC) (Sykam system Model S7130, Sykam GmbH, Eresing, Germany).

Amino acid apparent digestibility: The amino acid apparent digestibility was determined according to Sarwar *et al*. (1989) using the following equation:

$$\text{Apparent digestibility (\%)} = \frac{(\text{Amino acid intake} - \text{Fecal amino acid})}{\text{Amino acid intake}} \text{X100}$$

Statistical analysis: Each determination was carried at least six times and analyzed in triplicates, and figures were then averaged. Data were assessed by the analysis of variance (ANOVA) (Snedecor & Cochran, 1987), while mean values were compared by Duncan's Multiple Range test (P≤0.05).

RESULTS AND DISCUSSION

Table 2 shows food intake, weight gain, apparent digestibility of protein, fat and energy, true protein digestibility, nitrogen biological value and net nitrogen utilization of rats fed diets formulated with gum arabic, carageenan and cellulose as a control. As shown in Table 2, food intake was not significantly (P≤0.05) varied between the groups fed diet contains gum, carageenan and cellulose. The results indicated that food intake of rats did not affected by any source of dietary fiber. This due to the fact that rats received dietary protein will consume more food than those on a non-protein diet. Compared to cellulose as the control, the group fed diet formulated with gum Arabic slightly had high value of weight gain followed by diet containing carageenan and cellulose. The improvement in weight of diet formulated with gum Arabic could be attributed to the amount of food intake as well as utilization of nutrients mixed with gum. Compared to cellulose as the control, supplementation of a normal diet with gum Arabic or carrageenan did not influence weight gain significantly. Contrary to this finding Frias and Sgarbieri (1998) observed a decrease in food intake and body weight when rats fed diets containing 0, 10 and 20% guar gum. These finding and others suggested the use of water soluble fiber such as guar gum as a therapy for weight management. The animal group fed diet with gum Arabic showed significantly (P≤0.05) high level of nitrogen intake as well as nitrogen balance compared to other two groups but the level of nitrogen excreted in feces was high and that of urine was low compared to the other groups. The results of this study show that addition of fiber to the diet caused increased excretion of fecal nitrogen. It is also clear that the increased fecal nitrogen is due, in part at least, to fermentation of soluble fiber that stimulate microbial growth resulting in relatively high excretion of microbial protein together with undigested protein. The apparent protein digestibility of the group fed diet with CE (88.04) was significantly (P≤0.05) higher than that of groups fed diet with CR (84.99%) and slightly higher but not significant than group fed GA (86.61). Urbano and Goni (2002) reported that dietary fiber reduced the apparent digestibility of rat fed diet contained fiber compared to normal diets. Moreover, addition of dietary fiber of different levels to rat diets reduced the apparent digestibility of the protein as the level of fiber increased (Malkki and Virtanen 2001). On the other hand, the apparent digestibility of fat and energy of diet formulated with GA were 94.22% and 94.53%, respectively and were significantly (P≤0.05) higher than that of the groups fed diets formulated with CE and CR. Many studies had shown that the apparent protein digestibility is markedly reduced by viscous well fermented fiber compared to poorly fermented fiber (Eggum 1995). They further reported that soluble dietary fiber increases the viscosity and decreases the fluidity of intestinal fluids, thickens the unstirred water layer which nutrients must cross in order to be absorbed and they adsorbed and interferes with the interaction of digestive enzymes and substrates. The results revealed that addition of GA to the diets improved both fat and energy metabolism, this may be due to the stabilization of fat emulsion and/or stimulation of lipase activity by gum Arabic (Tsujita *et al*. 2007). As shown in Table 2, there was no significant (P≤0.05) difference in true protein digestibility among the groups but both diets formulated with CE and CR gave values higher than that of diet formulated with GA. No significant (P≤0.05) difference in net protein utilization and biological value among the groups with high value obtained for diet formulated with GA. The relationship between fiber and true protein digestibility, biological value and net protein utilization of the diets fed in this study indicated that GA is a useful source of fiber. Eggum (1995) reported that no effect of fiber on protein true digestibility, biological values and net protein utilization, they attributed that to the use of purified highly digestible protein and starch. The results of this study show that addition of fiber to the diet caused increased excretion of fecal nitrogen and thus a reduction in both apparent and true nitrogen digestibility. Several studies support the notion that the presence of dietary fiber in small bowl can impair protein utilization in addition other nutrients. Results obtained by different group have been contradictory in that some found decrease in protein utilization and others observed no differences. Some of the contradiction may have been due to the type of the fiber source that included in the diet. Eggum (1995) compared the results of rats, pig and human experiments and found that very similar conclusions could be drawn. Fiber, especially fiber associated with natural food sources, has negative effect on protein utilization, the influence depending greatly on structure and composition of fiber. The implications and mechanisms behind the effect of soluble and insoluble fiber on protein utilization are quite different. Hence soluble fiber is more fermentable and stimulates microbial growth resulting in relatively high excretion of microbial protein together with undigested protein. Furthermore the fermentation of soluble fiber in the large intestine produce short chain fatty acids that lower colonic pH, which convert toxic ammonia to the ammonium that is non-diffusible in portal blood system. The overall effect is an increase in fecal nitrogen secretion and a decrease in apparent protein digestibility. Table 3 shows energy of urine, gross fecal, gross intake and metabolites. The urine energy of the groups was found to be similar (0.03 Kcal/gm dry matter) and that of feces was slightly varied between the groups. The gross energy intake also slightly varied between the groups while the metabolic energy was slightly high for the groups fed CR (4.47 Kcal/gm) followed by those fed GA (4.42 Kcal/gm). Malkki and Virtanen (2001) reported a reduction in metabolic energy in rats fed diet contained oat bran compared to normal diets. Moreover, Baer *et al*. (1997) reported that addition of fiber to human diet reduced the digestibility of fat and protein and consequently reduced the metabolic energy. Generally the results showed that the both urine and fecal energy are low especially in groups fed GA containing diet which indicated that the animals make use of the energy sources and released small amount. The apparent amino acids digestibility of the groups fed three diets formulated either with CE, GA or CR is shown in Table 4. The amino acids digestibility of the group fed diet with cellulose ranged from 70.84 to 93.14% and that of diet with GA ranged from 73.59 to 96.79% while that of diet with CR ranged from 82.57 to 98.26%. It was observed that tyrosine, methionine, phenyle alanine, leucine and lysine were the most digestible amino acids (> 90%) in

Table 1. Ingredients (gm) of diets formulated with different sources of dietary fiber.

Ingredients	Diet supplement					
	Cellulose diet		Gum Arabic diet		Carageenan diet	
Casein	11.60	--	11.60	--	11.60	--
Egg protein	--	04.92	--	04.92	--	04.92
Sucrose	09.00	09.00	09.00	09.00	09.00	09.00
Vitamins mix	01.00	01.00	01.00	01.00	01.00	01.00
Minerals mix	03.50	03.50	03.50	03.50	03.50	03.50
Coheline	00.25	05.20	05.20	05.20	05.20	05.20
Methionine	00.10	--	00.10	--	00.10	--
Fiber source	05.20	05.20	05.20	05.20	05.20	05.20
Corn oil	05.20	05.20	05.20	05.20	05.20	05.20
Starch	64.15	70.93	64.15	70.93	64.15	70.93

Table 2. Food intake, weight gain, apparent protein, fat and energy digestibility, N biological value and N utilization in rat fed dietary fiber from different sources.

Parameter	Cellulose	Gum Arabic	Carageenan
Food intake (gm)	89.26±0.44[a]	89.28±0.29[a]	89.30±0.34[a]
Weight gain (gm)	22.37±1.28[a]	23.44±1.492[a]	22.55±0.88[a]
Apparent protein digestibility (%)	88.04±0.57[a]	86.61±0.43[ba]	85.00±1.49[b]
Apparent fat digestibility (%)	91.19±0.275[b]	94.22±0.30[a]	91.05±0.24[b]
Apparent energy digestibility (%)	89.08±1.23[b]	94.53±0.98[a]	91.43±2.34[ba]
True protein digestibility (%)	93.35±0.51[a]	93.62±1.09[a]	95.83±0.63[a]
Nitrogen biological value	86.01±2.60[a]	88.52±2.63[a]	85.49±1.23[a]
Net nitrogen utilization	82.00±0.024[a]	82.80±0.03[a]	81.90±0.02[a]

Values are means of six replicates ± SD. Means not sharing a common superscript(s) in a row are significantly different at $p \leq 0.05$.

Table 3. Metabolic energy (Kcal/gm DM) in rats fed dietary fiber from different sources.

Parameter	Cellulose	Gum Arabic	Carageenan
Urine energy	0.028±0.032[a]	0.025±0.033[a]	0.028±0.015[a]
Gross fecal energy	0.493±0.553[a]	0.437±0.457[a]	0.420±1.153[a]
Gross energy intake	4.518±0.130[c]	4.703±0.078[b]	4.922±0.088[a]
Metabolic energy	3.999±0.591[b]	4.421±0.503[a]	4.474±1.170[a]

Values are means of six replicates ± SD. Means not sharing a common superscript(s) in a row are significantly different at $p \leq 0.05$.

Table 4. Apparent amino acids digestibility of rats fed diets formulated with cellulose, gum Arabic or carageenan

Amino acid	Diet supplement		
	Cellulose	Gum Arabic	Carageenan
ASP	82.120±0.892[b]	76.865±0.898[c]	88.426±0.939[a]
GLU	86.858±0.608)[c]	88.745±0.494[b]	92.171±0.423[a]
SER	70.839±1.533[b]	73.595±1.314[b]	88573±0.657[a]
HIS	82.477±1.366[b]	96.788±0.102[a]	98.262±0.141[a]
ALA	76.054±1.181[b]	66.622±1.250[c]	82.572±1.401[a]
ARG	88.311±0.804[b]	83.867±0.676[c]	92.107±0.584[a]
TYR	93.140±0.517[b]	89.211±0.514[c]	95.981±0.460[a]
VAL	87.227±0.778[b]	83.581±0.597[c]	90.290±0.629[a]
MET	92.379±0.665[b]	87.565±0.352[c]	96.615±0.197[a]
PHE	93.078±0.490[b]	89.583±0.474[c]	94.941±0.386[a]
ILE	80.679±1.119[b]	78.693±0.738[b]	86.315±0.698[a]
LEU	91.955±0.496[b]	89.497±0.441[c]	94.541±0.361[a]
LYS	91.477±0.535[b]	88.63±0.408[c]	93.488±0.317[a]

Values are means of six replicates ± SD. Means not sharing a common superscript(s) in a row are significantly different at $p \leq 0.05$.

diet containing CE and that of diet containing GA only histidine gave value greater than 90% while those of diet with CR were glutamine, histidine, arginine, tyrosine, valine, methionine, phenyl alanine, leucine and lysine. It has been reported that the apparent digestibility of the amino acids depend primarily on type and level of dietary fiber (Eggum 1995). El Kossori *et al*. (2000) reported that the increased true ileal digestibility of the casein amino acids cannot be attributed to an increase in proteolytic enzyme activity in the small intestine, because the activities of trypsin, chymotrypsin and aminopeptidase-N in the small intestinal contents were not affected by changes in dietary fiber viscosity. It should be noted, however, that the results of enzyme assays give an indication of the total amount of enzyme present, following their isolation from the milieu of the intestinal digesta, where factors such as the partitioning of enzymes and solutes within the gel matrix, and slower rates of diffusion and mixing, may decrease the actual enzyme activity in the small intestine of rats fed more viscous fibers.

Conclusion: This study indicates that gum Arabic as a source of dietary fiber compared to carrgeenan and cellulose did not affect food intake and the growth rate of rats, improved the digestibility of the protein, fat and energy as well as the true protein digestibility. The gross energy intake and metabolic energies did not affected by addition of GA and were found to be higher than that of cellulose. Addition of GA improved the apparent digestibility of some amino acids.

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